phosphate consumed per molecule of lactate oxidized in cat heart extracts, Ochoa argued that in fact three molecules of ATP were formed per oxygen atom reduced (Ochoa, 1943). This P:O ratio was eventually accepted after considerable conflict.

The research on cellular respiration had been remarkably fruitful in the earlier decades of the twentieth century, with major advances achieved in the 1930s. Researchers had figured out the basic schema of the overall process, but also recognized many gaps in their knowledge. Figure 3.15 shows the understanding of the component mechanisms of glycolysis, the citric acid cycle, and electron transport as they were understood in the early 1940s, with question marks indicating points at which investigators recognized important gaps that still needed to be filled in.

The State of Biochemistry circa 1940

Biochemistry not only came into its own in the first four decades of the twentieth century but made impressive advances in understanding chemical processes in the cell. The Embden-Meyerhof pathway provided a comprehensive mechanism for glycolysis, and together the citric acid cycle and electron transport chain provided a very detailed sketch of the mechanism of aerobic cellular respiration. However, two problems were emerging for filling this in. First, researchers faced a challenge in explaining the linkage between the oxidation operations and phosphorylation. Based on the example of fermentation, they assumed that intermediate phosphorylated compounds formed along the electron transport chain that would transfer a high-energy phosphate bond to ADP. As I will discuss in Chapter 6, such compounds were never found. Second, biochemists were unable to carry out the entire reaction in a cell-free extract, a critical step if they were going to satisfy the biochemical standards for successful understanding of a biochemical reaction. That required isolation of the individual components (enzymes, cofactors, etc.) and resynthesis of a functioning system from them.[45] The problem, as Keilin and Hartree (1940) discovered, was that membranes seemed to be required in any preparation that carried out oxidative phosphorylation. The recognition of the role of membranes in oxidative phosphorylation was important, because

[45] Twenty-five years later Efraim Racker notes the failure to satisfy this demand as a problem facing the biochemistry of oxidative phosphorylation: "The mechanism of energy production in mitochondria has long defied analysis, since a complex chemical pathway in a living organism cannot really be understood until its intermediate products have been identified and the enzymes that catalyze each step of the process have been individually resolved as soluble components" (1968, p. 32).